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## AzureSeq One-Step Universal RT-qPCR Kit SARS-CoV-2 Instructions for Use – Direct no Extraction from iSWAB-Microbiome-EL

**Note: The AzureSeq protocol calls for heating sample for 5 minutes at 95°C, we found this step unnecessary when collecting with iSWAB-Microbiome-EL thus saving even more time.**

### **Sample processing procedure for Extraction-Less direct one step PCR-RT**

1. Obtain swabbed OP/NP material in iSWAB-Microbiome-EL

### **RT-qPCR Master Mix and Reagent Setup (AzureSeq SKU # AzureSeq-200, -1000, -2000, & -Custom)**

2. Completely thaw the CoVi Primer/Probe mix 3 (brown tube/cap) by setting on ice for ~30 minutes. Once thawed, briefly centrifuge to collect at the bottom of the tube, then add 367µL of nuclease free water to the tube. Mark the tube with water added.
3. Vortex the tube at max speed for 10 seconds to mix, then spin down briefly to collect at bottom of tube.
4. Proceed to master mix setup as shown below in a clean room or designated setup area:

**Reaction set-up for 20µL reaction volume Table 1:**

Component	Volume 1 Reaction	Volume 100 reactions (96-well plate)	Final Concentration
2X InhibiTaq Multiplex qPCR MasterMix	10 µl	1000 µl	1X
RTScript™, 200U/µL	0.5 µl	50 µl	5 units/µl
Diluted Primer/Probe Mix (Step #1)	4.5 µl	450 µl	1X

5. Mix the master mix by pipetting up and down repeatedly with pipette set to volume of 2X master mix added, or by capping the tube, vortexing briefly, and spinning down briefly to collect mix.
6. Distribute 15 µl of the master mix using an appropriate pipette to all wells of a plate that will be used.
7. Add 5 µl of collected sample directly from iSWAB collection tube, positive control, or negative control to appropriate wells.
8. Seal the plate, vortex briefly or flick to mix; spin down in a centrifuge to collect the mixed samples.
9. Place the plate into the designated real-time machine and run the following program:



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**Thermal cycling conditions:**

Recommended Cycling Conditions

Cycling Step	Stage	No. of Cycles	Temperature	Holding Time
RT Incubation	1	1	50°C	15 minutes
Enzyme Activation	2	1	95°C	2 minutes
Amplification**	3	45	95°C	3 seconds
			60°C**	30 seconds

\*\*Collect fluorescence during annealing/extension phase (55°C) step on FAM, HEX, and ROX channels (or equivalent channels).

**Risk and Safety Information:**

Safety Data Sheets are available online for AzureSeq at [www.seqonce.com](http://www.seqonce.com) and iSWAB-Microbiome-EL at [www.mawidna.com](http://www.mawidna.com)

The user should carefully read all warnings, instructions or Safety Data Sheets provided by the supplier for any additional materials or chemicals required for the use of the AzureSeq One-Step Universal RT-qPCR Kit SARS-CoV-2 and iSWAB-Microbiome-EL. The user should also follow general safety precautions when handling biohazards, chemicals and other materials.

**General Precautions:**

1. Treat all samples, materials and instrumentation as potentially infectious.
2. Avoid potential contamination by employing good laboratory practices, wearing proper personal protective equipment, and decontaminating workspaces before and after use.
3. All instruments must be maintained and operated according to manufacturer's instructions.
4. Dispose of waste according to state and local regulations.
5. Only use DNase and RNase free consumables.
6. Do not use reagents beyond their expiration.